

## Study the inhalation exposure effect of pesticides mixture in the white rat

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**Abstract:** The present work was aimed to evaluate the risk of inhalation exposure of chlorpyrifos and cypermethrin mixture which is recently formulated and sale ready to use in Egypt especially most of the pesticide applicators in Egypt are not protected by safety measures when handling with the pesticides. The study was included some toxicological assays ranged from cytogenetic, histopathological, and oxidative stress assay. Twelve healthy male white rats weighing  $140\text{g}\pm 10\%$  were used and divided into three groups (a, b, and c) each of four rats per cage. The first group (a) was kept as control without exposure to any pesticides just a fresh air. Group (b) was exposed to low inhalation dose ( $44\text{mg/L}$ ), group (c) was exposed to high inhalation dose ( $120\text{mg/L}$ ). Inhalation chamber used was supplied with two fans for inlet and outlet airflow and in one side supplied with vial containing the pesticide tested and tissue paper to help of spreading the vapor of the pesticide. The results revealed that, the inhalation exposures for 28 consecutive days induce highly significant effect ( $p < 0.001$ ) at high exposure dose and slight significant effect ( $p < 0.05$ ) at low exposure dose in both genotoxicity and cholinesterase assays. Also induced highly significant effect ( $p < 0.001$ ) in lipid peroxidation at high exposure dose and slight inhibition in the antioxidant enzymes Catalase and reduced in glutathione contents only at high dose but at low exposure dose no significant effect recorded. On the other hand the histopathology of lung tissue found to be severe changes by exhibition fibrosis with collagen proliferation and aggregation of the lymphoid cells in peribronchiolar tissue associated with sever dilatation and congestion of the blood vessels and hyperplasia in bronchiolar and lining epithelium with polyps formation at high exposure dose. Despite existing findings based on study with experimental animals it cannot be predicted to what extent of pesticides inhalation exposure will affect on humans because the rats are more tolerant than human and these results alarm of this type of exposure and strongly impose the need to more detailed testing of the toxicity of occupational exposure to pesticides especially by inhalation and the caution should be exercised in their handling in prolonged exposure time that may lead to adverse health effects.

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### 1. Introduction:

The large volume of pesticides used in agriculture necessitates evaluation of the potential adverse effects on agricultural workers' health. The large scale use of mixtures of pesticides has been increased in developing countries (**Committee on Toxicity of Chemicals, 2002**). The introduction of these new mixtures into the environment has necessitated accurate identification of their potential hazards to human and animal health (**Bolognesi, 2003**). Furthermore, the local manufacturers formulate a mixture of pesticides ready to use. These mixtures may be more hazards than using each of their alone. On the other hand the occupational exposure to pesticides has been linked to the human body by three ways: dermal through the skin or eyes, oral through the mouth, respiratory or inhalation through the lungs (**British Colombia, 2013**).

The last route of exposure by inhalation may be accidental route of entry in Egypt because the Egyptian spray workers do not care to use the safety measures of pesticides. Furthermore, the exposure by inhalation is very hazardous because the lungs can rapidly absorb the pesticides into bloodstream. Some pesticides can cause serious damage to the nose and lung tissue if inhaled in sufficient amounts. Vapors and very small particles pose the most serious risks. Also lungs can be exposed to pesticides by inhalation of powders, airborne droplets, or vapors. In addition concentrated wettable powders can pose a hazard if inhaled during mixing. Many pesticides have been tested for the toxicity of oral and dermal treatments. A limited number of these studies have been done to evaluate the risk of inhalation exposure, especially most of the pesticide workers in developing countries and Egypt are not protected by safety measures when using the pesticides in the fields; therefore they are

directly exposed to these compounds, whose toxicity is ranged from moderate to hazardous (**Mansour, 2004**).

A new pesticide formulation (Chloroplus 29% EC) local made was used in our study. It is sale as a pesticide ready to use in agricultural pest control in Egypt. This mixture may be included some second products or impurities which are unknown. So study the toxicity of this new mixture is very essential and necessary for risk assessment. The pesticide formulation consists of two traditional pesticides chlorpyrifos and cypermethrin. Chlorpyrifos is one of organophosphate (OP) insecticides which are still widely used for agriculture, pest control and domestic purposes. Thus the uncontrolled application of these insecticides in agriculture and public health operations increased the scope of ecological imbalance and thus many non-target organisms have become victim (**Ojha et al., 2011**). Organophosphate (OP) compounds including Chlorpyrifos are known to inhibit acetyl-cholinesterase and pseudocholinesterase in target tissues resulting in accumulation of acetylcholine in synaptic junctions (**Shenouda et al., 2009**). This excessive accumulation of acetylcholine in synapses leads to activation of cholinergic, muscarinic and nicotinic receptors and hyperactivity in the cholinergic pathways. However, AChE inhibition does not explain all the symptoms of OP intoxication. Other systems that may be affected by OP exposure are the immune system (**Galloway and Handy, 2003**). Hematological system (**Jintana et al., 2009**), and reproductive system (**Farag et al., 2000 and Uzun et al., 2009**). OP pesticides have demonstrated genotoxic, alkylating and clastogenic properties; thus they are potentially mutagenic and clastogenic (**Mehta et al., 2008**). More recently, it has been postulated that OP pesticides produce oxidative stress in different tissues through the formation of reactive oxygen species (ROS) (**Akhgari et al., 2003; Abdollahi et al., 2004; and Mehta et al., 2009**).

On the other hand cypermethrin is widely used as insecticide in developing countries to control a wide range of insects, especially Lepidoptera. It is recently back to use in agriculture after it banned for several years in Egypt. Human exposure to cypermethrin is reported to occur mainly occupationally during application or through pyrethroids residues such as those detected in cow's milk, bread, fruits and vegetables (**Sankar et al., 2010**). In an epidemiological study, population exposed to cypermethrin in cotton fields showed ill health effects such as severe giddiness, nervous, skin and eye disorders, neonatal deaths and congenital defects. Cypermethrin can also elicit a range of neurotoxic, immunotoxic and genotoxic effects and reproductive toxicity in various experimental systems

(**Yousef et al., 2003**). Cypermethrin caused an increase in the number of cells with abnormal chromosomes in both bone marrow and spleen (**Institoris et al., 1999**). All of the above mentioned make cypermethrin is one of the most common contaminants in the ecosystem.

Both pesticides tested are commonly used in formulation form emulsifiable concentrate (EC). This type of formulation has high vapor pressure so, the current study was undertaken in order to recognize the inhalation exposure effect of this mixture of pesticides for consecutive 28 days and evaluate the changes in the histology of rat lung and its effect on some toxicological parameters included cytogenetic and oxidative stress.

## 2. Materials and Methods

### 2.1 The pesticide formulation tested

The pesticide formulation tested consists of cypermethrin 5%, the IUPAC name: (*RS*) -  $\alpha$ -cyano-3-phenoxybenzyl (1*RS*, 3*RS*; 1*RS*, 3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate. And chlorpyrifos 24%, the IUPAC name: (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphorothioate). The formulation was supplied as well by the Department of Mammalian and Aquatic Organisms Toxicity Research, Central Agricultural Pesticides Lab, Dokki, Egypt. The formulation was used as it is without diluted.

### 2.2 Animals

Fifteen healthy male white rats (*Rattus rattus*) of Wistar strain weighing 140g $\pm$ 10% were used throughout the whole work. The animals were obtained from the laboratory animal house of the Modern Veterinary Office, Giza, Egypt. Animals were kept under full hygienic conditions, had free access to fresh water and fresh well-balanced food, and remained under supervision for two weeks before commencing the experimental work. The animals were housed in all groups of five rats per cage.

### 2.3 Experimental design

Rats were divided into three groups (a, b, and c) each of four rats per cage. The first group (a) was kept as control without exposure just a fresh air. Each of group (b) and (c) were used in inhalation treatments. Group (b) was exposed to low dose (44mg/L), group (c) was exposed to high dose (120mg/L). We used inhalation chamber which is local made and consists of plastic chamber with two fans for inlet and outlet airflow and supplied in one side with vial contained the pesticide tested and tissue paper that help to spread the vapor of the pesticide. This chamber is suitable for liquid formulation only. The inhalation chamber is illustrated in figure (1) which clears the design and working process of it.

### 2.5 Blood collection and tissue preparation

Rats were sacrificed through cutting of their neck veins at the end of treatments after they were anaesthetized by ether. Blood samples were collected from the sacrificed animals and placed immediately on ice. Heparin was used as an anticoagulant and plasma samples were obtained by centrifugation at 1,100 rpm for 20 min and plasma samples were used

immediately for analyzing the oxidative stress parameters. Lung was immediately removed and washed using chilled saline solution. Then tissues were preserved in formalin (10%, w/v) for histological parameter. Both femurs were dissected out and prepared for chromosomal aberrations assay.

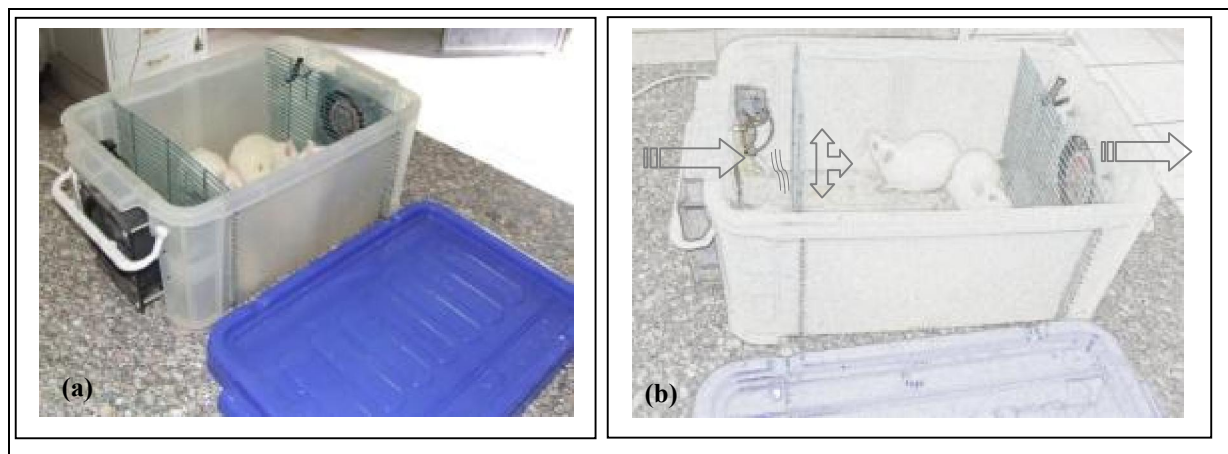


Fig (1) the inhalation chamber(a), and the pathway of inlet and outlet of airflow(b)

## 2.6 Biochemical parameters

### 2.6.1. Glutathione Reduced (GSH)

The method based on the reduction of 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. Briefly fresh heparinized blood sample was used and the erythrocytes (red blood cells) were lysed by add 4 fold volume cold distilled water then centrifuged at 4,000 rpm for 15 min. the supernatant was collected and added to the reagent contain (DTNB) then mixed well and measured the absorbance after 5-10 min. the method according to (Beutler and Kelly, 1963).

### 2.6.2. Catalase (CAT)

Catalase (CAT) activity was estimated by the method of (Aebi *et al.*, 1984). The method based on the Catalase reacts with a known quantity of  $H_2O_2$  and the reaction is stopped after exactly one minute with catalase inhibitor. Briefly the plasma were collected as above mentioned and added to the reaction mixture containing 0.8 ml phosphate buffer ( $K_2HPO_4/NaH_2PO_4$ , 50 mM, pH 7.0), and 0.1 ml triton X-100 (0.02%) then incubated at room temperature for 10min. Reaction was initiated by addition of 2.0 ml  $H_2O_2$  (0.03 M prepared in potassium phosphate buffer, pH 7.0) and absorbance change per min was recorded

for 5min at 240nm. Specific activity is expressed as 1 mole  $H_2O_2$  decomposed  $min^{-1} mg^{-1}$  protein.

### 2.6.3. Lipid peroxidation

The end-products of lipid peroxidation, is malondialdehyde (MDA) which reacts with thiobarbituric acid reactive substances (TBARS) to yield a fluorescent product so the most commonly used test is called (TBARS) assay (Okhawa *et al.*, 1979). Thiobarbituric acid (TBA) reacts with (MDA) in acidic medium at temperature of  $95^\circ C$  for 30min to form thiobarbituric acid reactive product, the pink product can be measured at 534 nm. A 10% (w/v) of liver tissue prepared in 1.15% KCl was used as a homogenate sample in this assay. The levels of MDA are expressed as  $nmol g^{-1}$  tissue.

### 2.6.4. Plasma cholinesterase

Plasma cholinesterase or butyrylcholinesterase (BuChE) activity was estimated by the method of (Ellman *et al.* 1961). The reaction mixture containing 2.6 ml sodium phosphate buffer (0.1 M, pH 7.5), 0.15 ml of DTNB (dithio-bis-2-nitrobenzoic acid, 10 mM, pH 7.0; containing 3 mg  $NaHCO_3$  per 8 mg DTNB) and 0.1 ml plasma was incubated at room temperature for 10 min. Reaction was initiated by addition of 0.15 ml acetylthiocholine iodide (12.5 mM) and absorbance change per min was recorded at 412nm for 4min. Specific activity is expressed as nmoles product formed  $min^{-1} mg^{-1}$  protein using molar

extinction coefficient of the adduct formed between thiocholine and DTNB as  $13600 \text{ M}^{-1} \text{ cm}^{-1}$ .

### 2.7. Histological section preparation

Liver specimens were obtained from rats, and immediately fixed in 10% formalin for 24 hr and decalcification was occurred on formic acid then washed in tap water. Serial dilutions of alcohol (absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at  $56^\circ\text{C}$  in hot air oven for 24 hr. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by Hematoxylin & Eosin stain then examination was done through the light microscope (Banchroft *et al.*, 1996).

### 2.8 Cytogenetic investigations

Cytogenetic analysis of rat chromosome aberrations (CA) in bone-marrow metaphase cells was performed according to the technique described earlier by (Adler, 1984) with some modifications as recommended by authors. Animals were intra-peritoneal injected with colchicines (4mg/kg bw) 2 hrs prior to the scheduled time of sacrifice, in order to accumulate metaphase cells and provide more readily analyzable chromosomes. The rats were sacrificed 24h after the last dose treatment. Both femurs were dissected out and cleaned of any adhering muscle, the bone-marrow cells were collected from both femurs by flushing with Hank's balanced salt solution (HBSS) then centrifuged at  $101\times g$  for 10min and the was re-suspended very well with a potassium chloride hypotonic solution (0.075 M KCl) and after that the tubes were kept in the refrigerator at  $4^\circ\text{C}$  for 45-min. This step is very important (modified and recommended by the authors). The cell suspension was

centrifuged at  $101\times g$  for 10min, fixed in methanol: glacial acetic acid, 3:1, v/v). Centrifugation and fixation (in the cold) were repeated three times at 20min intervals. The material was re-suspended in a small volume of fixative, dropped on to chilled slides, flame-dried, and stained in the next day with 20% Giemsa. One hundred good-quality metaphases containing 42 chromosomes were examined per animal to score different aberrations.

### 2.9 Statistical analysis

The data obtained in this study were calculated and statistically analyzed, according to Student's t-test (Venables and Ripley, 2002), using statistical software.

## 3. Results

### 3.2. Effects of the tested pesticide on the cytogenetic parameters

Structural chromosomal aberrations observed in the present study were in the form of chromatid gap (tg), end to end association (E-E), centromeric attenuation (C-A), and chromosome association (C-S). The numerical chromosomal aberration was observed in the form of polyploidy (4n). A significant ( $P < 0.001$ ) in the total aberrant cells at high dose exposure was scored when compared with that scored in the control group. In the low dose exposure, the increase in the number of aberrant cells was slight significant ( $P < 0.05$ ). On the other hand the most frequent aberrations were centromeric attenuation (C-A) and end to end association (E-E), while the chromatid gap (tg) and chromosome association (C-S) were the lowest aberrations scored. Numerical aberrations (polyploidy) were recorded in the negative control group but the treatments increased the percentage of their.

**Table(1): Chromosomal aberrations induced in rats bone marrow after inhalation exposure of the pesticide tested**

Treatments	Types of structural aberrations				Numerical aberration (polyploidy)	Total aberrant cells/ 400 scored metaphases	Mean $\pm$ S.E.
	tg	E-E	C-A	C-S			
<sup>a</sup> Control group	2	1	2	0	5	10	$2.5 \pm 0.645$
Group (b) Low exposure dose	6	2	6	2	7	23	$5.7 \pm 1.031$ *
Group (c) High exposure dose	5	8	8	6	11	38	$9.5 \pm 0.500$ ***

Values are from four replicates in each treatment and the last column represent mean  $\pm$  S.E. of aberrant cells per 400 spread metaphases/treatment, \*\*\* Significant at  $p < 0.001$ ; \* Significant at  $p < 0.05$

<sup>a</sup>Control group, the rats in inhalation chamber were exposed to a fresh air only

Abbreviations: **tg**, chromatid gap; **tb**, chromatid break; **E-E**, end to end association; **C-A**, centromeric attenuation; **C-S**, chromosome association



### 3.3. Effects of the pesticide tested on antioxidant parameters

The results summarized in Table (2) indicated the rats were exposed to the pesticide tested increased significantly the level of TBARS ( $p < 0.001$ ) at high exposure dose only as indication of increased the lipid peroxidation but the low exposure dose did not induce significant change. The GSH content significantly decreases only at high exposure dose ( $p < 0.01$ ). Also the activities of CAT enzyme were significantly slight decreased ( $p < 0.05$ ) at high dose. On the other hand, the pesticide tested decrease the level of plasma cholinesterase or butyrylcholinesterase BuChE ( $p < 0.001$ ) at high exposure dose and slightly decrease ( $p < 0.05$ ) at low exposure dose.

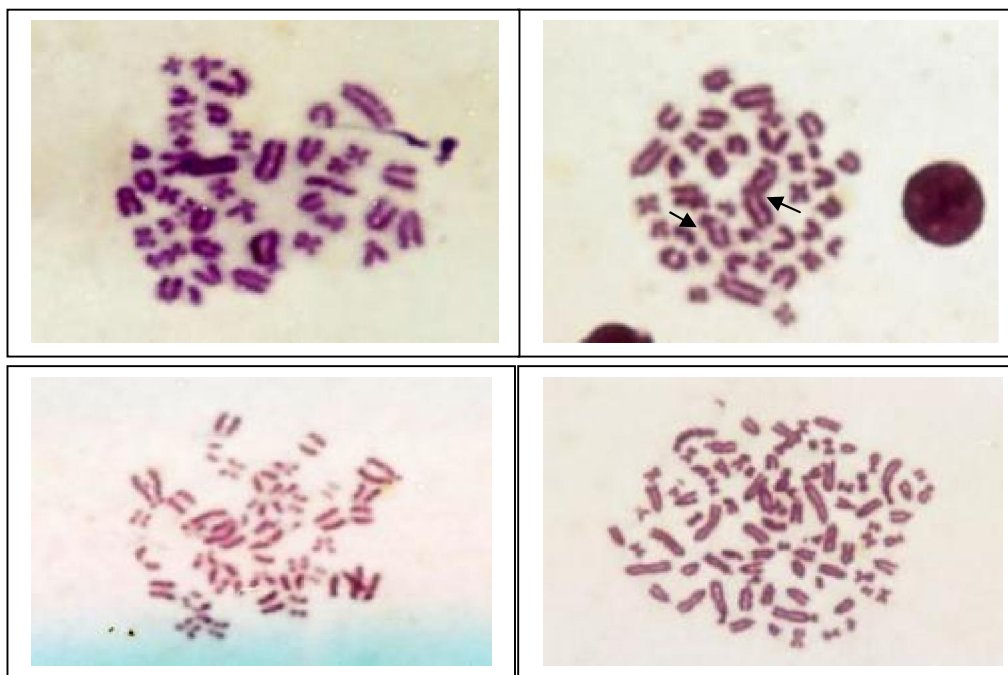


Fig (2) Rats bone marrow metaphases, showing (a) chromatid gap; (b) chromosome association; (c) centromeric attenuation; and (d) polyploidy(4n).

Table(2):The activities of CAT, BuChE, and the levels of GSH in blood and TBARS in liver of rats after inhalation exposure of tested pesticide

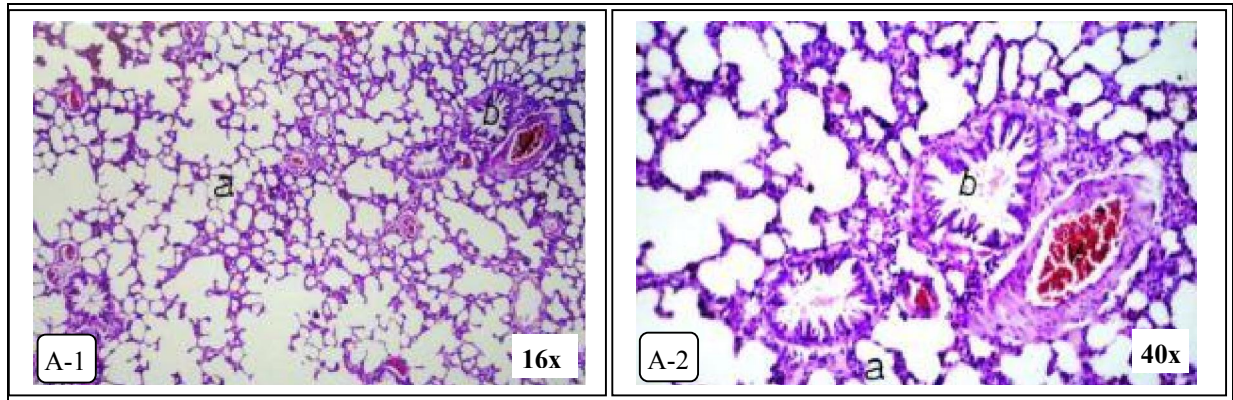
Parameters	Experimental groups		
	Control group	Group (b) Low exposure dose	Group (c) High exposure dose
TBARS (mg/g.tissue)	7.63± 0.24	8.63±0.42	10.05± 0.32 ***
GSH(mg/dL)	16.51±0.96	14.38±0.52	12.493 ±0.46 **
CAT (U/L)	446.62±22.39	409.38±2.663	377.582±1.011 *
BuChE (U/L)	374.1± 8.93	335.44±9.33 *	247.05±8.47 ***

\*\*\* Significant at  $p < 0.001$ ; \*\* Significant at  $p < 0.01$ ; and \* Significant at  $p < 0.05$

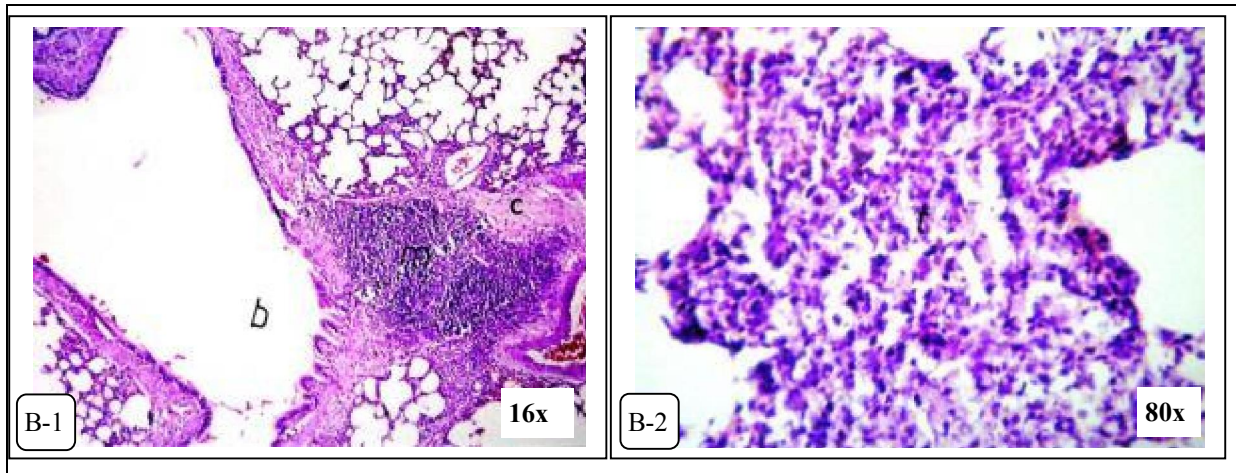
### 3.5. Histological examination of the liver

Histopathological examination of specimens taken from control group showed normal histological structure of the bronchiole, peribronchiolar blood vessels and air alveoli (Fig.3). But the specimens taken from exposed group showed fibrosis with collagen proliferation and focal proliferation and aggregation of the lymphoid cells in peribronchiolar tissue (Fig.4), associated with sever dilatation and congestion of the blood vessels (Fig.5).

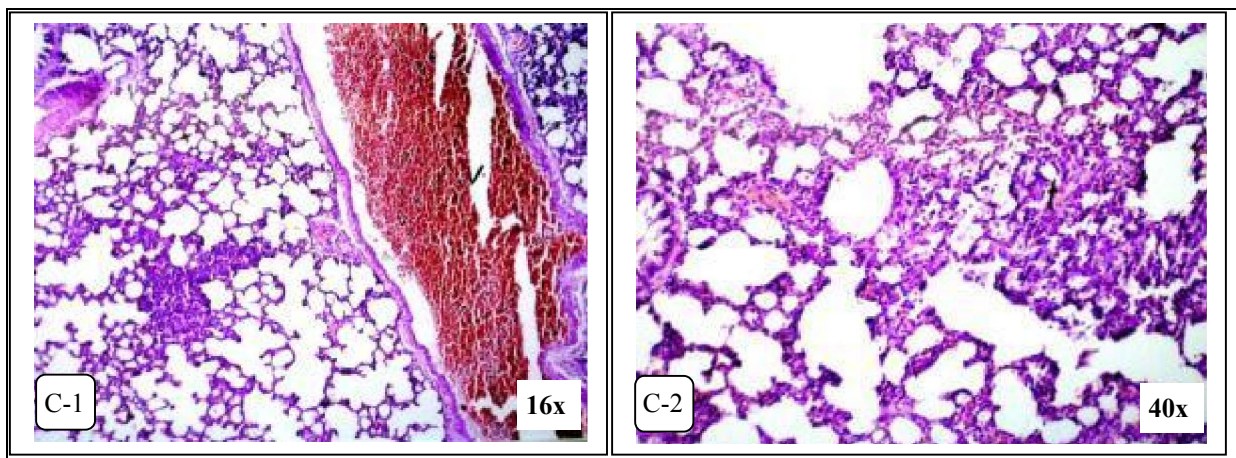
The air alveoli showed focal area of collapse by epithelization in the lining epithelial cells associated with compensatory emphysema in other while the bronchiolar lining epithelium showed hyperplasia with polyps formation (Fig.6).



**Fig (3).** Histopathology of lung tissue from control rats showing in (A-1 )normal histological structure of the bronchiole(a), peribronchiolar blood vessels(b) and air alveoli, (A-2) the magnification of area (b) & (v)

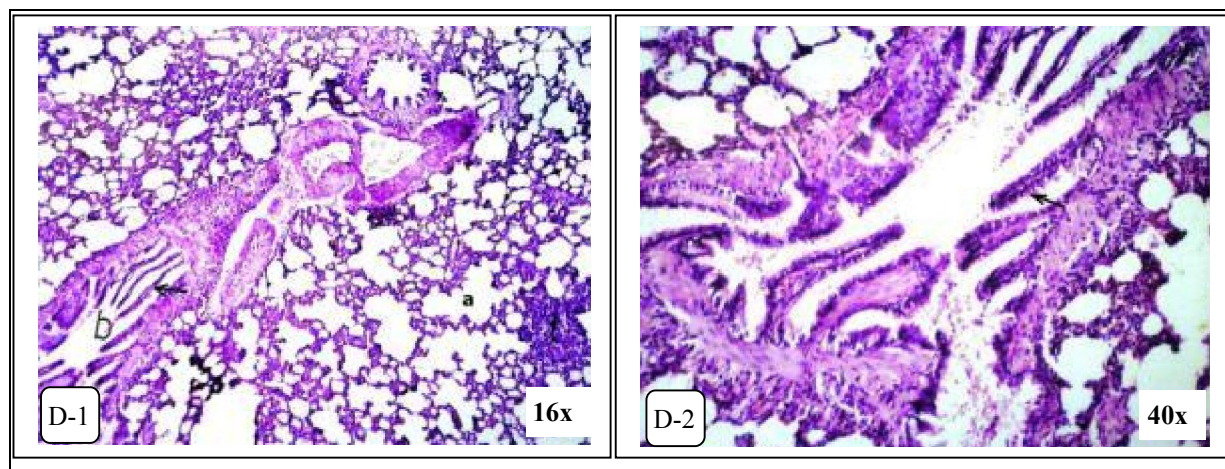


**Fig (4).** Histopathology of lung tissue from rats inhaled with low exposure dose showing in (B-1) peribronchiolar collagen proliferation (b), thickening(c), and lymphoid cells proliferation and aggregation (m) and showing in (B-2) the epithelization and collapse of the air alveoli (t)



**Fig (5).** Histopathology of lung tissue from rats inhaled with high exposure dose showing in (C-1) sever congestion and dilatation of blood vessels(v), and showing in (C-2) the epithelization and collapse of the air alveoli in lining epithelium (t)





**Fig (6).** Histopathology of lung tissue from rats inhaled with high exposure dose showing in (D-1) focal alveoli epithelization and collapse (t), with other emphysema(a), hyperplasia in bronchiolar(b), and lining epithelium with polyps formation (←) and showing in (D-2) the magnification of area (b) to identify the bronchiolar epithelial cells hyperplasia and polyps formation (←).

#### 4. Discussion

##### 4.1. Effect on the lung tissue

When toxic amounts of Ops are inhaled, the first effects are usually respiratory system and may include bloody or runny nose, and wheezing due to constriction or excess fluid in the bronchial tubes (EXTOXNET, 1993). There are very few reports on lung function following inhalation exposure of Ops in man. In high inhalation dose of Ops poisoning, both obstructive and restrictive lung dysfunction are reported (ACGIH, 1986). Respiratory effects of high-dose OP exposure include broncho-constriction, pulmonary oedema and respiratory muscle paralysis (Berg, 1986). In focus on chlorpyrifos the inhalation LC50 for chlorpyrifos in rats after 4-hour is greater than 200mg/L (Dow, 1992). Recently research indicated among 50 farm pesticides studied, chlorpyrifos was one of two chlorpyrifos and diazinon found to be associated with higher risks of lung cancer among frequent pesticide applicators than among infrequent or non-users and the study indicates a likely link between chlorpyrifos application and lung cancer (Rauh *et al.*, 2006). More addition the children who their mothers were exposed to chlorpyrifos during the pregnant had born with increased risk of delays in mental and motor development at age 3 and an increased occurrence of pervasive developmental disorders (IA, 2006). The obtained results of lung histopathology confirm these findings by exhibition fibrosis with collagen proliferation and aggregation of the lymphoid cells in peribronchiolar tissue associated with sever dilatation and congestion of the blood vessels

On the other hand, cypermethrin can enter commonly the body either by inhalation of air containing cypermethrin vapours, or by dermal

contact. Inhalation of cypermethrin can lead to inflammation of the lungs (pneumonitis), breathing difficulties (dyspnea), chest pain, wheezing and in extreme cases respiratory failure (Sheikh and Javed, 2009). More addition exposure to cypermethrin for time induced pulmonary edema, alveolitis and pulmonary fibrosis by the deposition of collagen. But exposure for long time resulted in the development of skin tumor in the epidermis of mice (Kehrer, 1995). Other reports concluded that cypermethrin and other pyrethroids cause hazardous effects in non target organisms through inhalation exposure (Grewal *et al.*, 2010) The observed findings suggested that cypermethrin produced irritant effect on the pulmonary tissue, because proliferation of fibroblasts located in the pulmonary interstitium frequently follows injury and continuous irritation. So, the use of this insecticide should be controlled seriously.

##### 4.2. Effects on the cytogenetic parameter

Genotoxic effects are considered among the most serious of the possible side effects of agricultural chemicals. The prolonged exposure to such chemicals may lead to effects including heritable genetic diseases, carcinogenesis and birth defects (Patel *et al.*, 2007). In spite of many previous findings reported that there is no indication of genotoxic activity for chlorpyrifos in many genotoxicity assays in both mice and rats (Gollapudi *et al.*, 1995) our data showed significant increased in (CAs) at high inhalation dose and low significant at low inhalation dose, this may be due to that one of the components of the pesticide tested has genotoxic effect than other. The finding that support this investigation the other component of the pesticide mixture, cypermethrin which has hydrophobic nature and small molecular size can passes through the cell membrane and reaches the

nucleus. It is suggested that within the nucleus it binds to DNA through the reactive groups of its acid moiety, leading to destabilization as well as unwinding of the DNA, which could be a possible mechanism for its genotoxicity (Saxena *et al.*, 2005). Moreover, cypermethrin has both a vinyl and a dimethylcyclopropane group. Dimethylcyclopropane may be oxidized into methyl butenol via the rearrangement of radical and the formation of carbocation (carbonium ion). If the vinyl group and/or the methyl butenol group undergo epoxidation, the resultant active metabolites may cause DNA damage (Sankar *et al.*, 2010). Cypermethrin has also been shown to induce oxidative stress and generation of reactive oxygen species (ROS) in experimental systems. It has been demonstrated that ROS may cause DNA damage, which could lead to single-strand breaks and mutation. In addition the difference in potential mutagenicity of cypermethrin *in vitro* and *in vivo* studies could be due to the fact that under *in vivo* system metabolites generated could be more reactive to DNA leading to increased genotoxicity (Patel *et al.*, 2007). Also (Gabbianelli *et al.*, 2004) suggested that superoxide anion and hydrogen peroxide are the main source of cypermethrin-induced free radical production, and this is coincided with the present results. This provides another probability that DNA damage observed after cypermethrin exposure could be a consequence of free radical attack. Thus, the increase in lipid peroxidation and depletion of antioxidant enzymes play a major role in cypermethrin-induced genotoxicity.

#### 4.3. Effects on the Oxidative stress parameters

Oxidative stress is unbalance between the productions of free radicals and antioxidant defenses in the body; it can result in oxidative damage to lipids (lipid peroxidation), proteins, carbohydrates, and nucleic acids. In most cases, the abnormal generation of reactive oxygen species (ROS), which results in significant damage to cell structure, is considered an important signal of oxidative damage (Barzilai and Yamamoto, 2004). Several xenobiotics and environmental pollutants are known to cause this imbalance between formation and removal of ROS.

Results of the current study showed that the combination of two active ingredients in the pesticide tested generated oxidative stress in rat tissues only at high inhalation dose except cholinesterase parameter. This is evidenced by the elevation in TBARS, the reduction in the antioxidant enzymes (CAT) and glutathione (GSH) content in rat tissue. This might be attributed to the metabolic activation of cypermethrin, which is considered a major mechanism of its toxicity. Cypermethrin caused significant oxidative stress in liver tissue of rats as was evident by the elevation of the level of TBARS. Reduced level of total

glutathione also indicated the occurrence of an oxidative insult (Giray *et al.*, 2001). Cypermethrin can be expected to have two modes of action: it may induce oxidative stress by generate (ROS) or, as a hydrophobic compound it may accumulate in cell membrane and disturb membrane structure.

On the other hand, OPs are known to produce oxidative stress by increased generation of ROS and decreased levels of cellular antioxidants (Saxena, 2010). The ROS may be produced as a result of the metabolism of Ops by cyt P450. The cyt P450s are monooxygenases and catalyze oxidation by addition of one atom of molecular oxygen into the OP compounds at electron transport pathway. Also OP pesticides induced peroxidative damage of membranes and accumulation of lipid peroxidation products in cells, tissues and serum of rats, these have been reported by (Institoris *et al.*, 1999) and (Sankar *et al.*, 2010).

More addition, Antioxidant enzymes namely catalase (CAT), and other enzymes are the first line of defense against oxidative stress. It catalyzes the decomposition of hydrogen peroxide to water and oxygen (Chelikani *et al.*, 2004). It is a very important enzyme in reproductive reactions. Likewise, catalase has one of the highest turnover numbers of all enzymes; one catalase molecule can convert millions of molecules of hydrogen peroxide to water and oxygen each second (Goodsell, 2010). Decrease in the activity of this enzyme change the following induction of oxidative stress. The results of the present study showed slight decrease in the activities of CAT enzyme in plasma of rats exposed to high inhalation dose. Furthermore, in healthy cells and tissue, more than 90% of the total glutathione pool is in the reduced form (GSH) and less than 10% exists in the disulfide form (GSSG). The ratio of reduced glutathione to oxidized glutathione within cells is often used as a measure of cellular toxicity and oxidative stress. So glutathione protects cells from the free radicals produced through oxidation (Pastore *et al.*, 2003). Our results showed slight significant decrease in (GSH) contents also at high inhalation dose but low inhalation dose did not induce significant decrease.

#### 4.4. Effect on the cholinesterase parameter

The pesticide tested is included chlorpyrifos which is organophosphate compound (OP) and these compounds are known to inhibit acetylcholinesterase or butirilcholinesterase (BuChE) in plasma so the inhibition of this enzyme is the admitted result when toxic amounts are inhaled Plasma cholinesterase levels activity has been shown to be inhibited when chlorpyrifos particles are inhaled [1] The results of the present study showed inhibition of BuChE activity.



There was a decrease in activity at both low and high doses. The level of inhibition was dose dependant.

### 5. Conclusion

The present study concluded that, the exposure to mixture of pesticides by inhalation for 28 consecutive days may induce moderately effect in genotoxicity and neurotoxicity but it induced severe histological changes in lung tissue. Also resulted in lipid peroxidation, inhibition in the antioxidant enzymes Catalase and reduced in the glutathione contents as indication of oxidative stress effect. Despite existing findings based on study with experimental animals it cannot be predicted to what extent of pesticides inhalation exposure will affect to humans because these animals are more tolerant than human. But the results still the alarm of this type of exposure. Finally, the results strongly impose the need to more detailed testing of the toxicity of pesticides mixture exposure especially by inhalation and the caution should be exercised in their handling as prolonged exposure time that may lead to adverse health effects

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